Preliminary Systematic Studies of the Oak Ferns: Chromatography and Electrophoresis

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The Oak Ferns, represented in the United States by *Gymnocarpium dryopteris*, *G. robertianum*, and their putative hybrid, have been placed in many different genera. Some authors have placed them in the genus *Dryopteris*, primarily on the basis of similarities in rachis characters and rhizome scaliness. Others have included them with the Beech Ferns (*Phegopteris*). The Beech Ferns and the Oak Ferns have in common an elongate creeping rhizome and exindusiate sori (Morton, 1950). Slosson placed them both in the cosmopolitan genus *Thelypteris*, along with the Marsh Ferns (Wherry, 1961, p. 64). Ching (1933) proposed that the Oak Ferns be regarded as a genus distinct from *Phegopteris*, and he redefined *Gymnocarpium*. Currently most workers appear to favor this interpretation; however, other combinations are frequently used, particularly in local and regional floras.

Cytological studies have shown a similarity in chromosome size between the Oak Ferns and the Beech Ferns; however, the number differs. The base number of 40 is found in the Oak Ferns (Wagner, 1966). The Broad Beech Fern and the Long Beech Fern have numbers of 30 and 90, respectively (Britton, 1965; Manton, 1950, p. 184). True *Dryopteris* has a base number of 41 chromosomes (Walker, 1961; 1962). These chromosomes tend to be somewhat larger than those of either the Beech or the Oak Ferns. Chromosome numbers vary within the genus *Thelypteris*; however, none reported correspond with that of the Oak Ferns.

In recent years paper chromatography has gained acceptance as a taxonomic method. Most of the studies have involved taxa at or below the species level. The works of Smith and Levin (1963), Alston and Turner (1963), and others have indicated chromatography to be of particular value in the confirmation of interspecific hybridization. Works with higher taxa have concerned primarily the distribution and occurrence of specific compounds (Kupchan et al. 1961; Mabry et al. 1963).

Cellulose acetate electrophoresis has been used in animal taxonomy (Leviton et al. 1964). No reports were located of comparable comparative studies in plants, though immunoelectrophoresis studies have been done with various plant taxa (Gell et al. 1956).

This study was undertaken to add further information concerning the status of the Oak Ferns by the consideration of biochemical characteristics, and to explore the value of paper chromatography and cellulose acetate electrophoresis in generic problems.

Chromatographs and electrophoretographs of extracts from the Oak Ferns were compared with those of representatives of the Beech Ferns, the Marsh Ferns (*Thelypteris*), and the Wood Ferns (*Dryopteris*).

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METHODS AND MATERIALS

Extensive collections of the ferns were made throughout Indiana, Ohio, Minnesota, and Wisconsin. Voucher specimens were deposited in the Ball State University Herbarium. Two populations of *Gymnocarpium robertianum* were sampled. The remaining species were represented by ten populations each. Twenty-five chromatograms and ten electrophoresis membranes were prepared for each population.

Extracts were prepared by powdering dried fronds and soaking the materials in methanol : hydrochloric acid : water (7.9 : 0.1 : 2.0) for 48 hours. Paper chromatograms were prepared by applying fifty microliters of each sample to Whatman #1 paper using the spot method. The chromatographs were run descendingly using butanol : acetic acid : water (12 : 3 : 5) as the solvent.

Dried chromatograms were examined in the presence of long wave ultraviolet light and ammonia vapor. The chromatograms were sprayed with ninhydrin for the detection of free-amino acids and related substances, and alkaline silver nitrate, a general reagent for the detection of phenolic compounds (Smith, 1958). Average rf values were calculated for the spots detected.

Electrophoresis studies were made using a Buchler migration chamber and Buchler cellulose acetate strips measuring one by six inches. Ten microliters of extract were applied with a streaking pipette at the center of each strip and migrations were allowed to proceed for one hour. The electrophoretic runs were conducted at 200 volts and five milliamperes in a double veronal buffer of pH 8.6. Electrophoresis patterns were observed under of long wave ultraviolet light.

Extracts of *Gymnocarpium dryopteris* and *G. robertianum* were compared chromatographically and electrophoretically with those of *Phegopteris polypodioides, P. hexagonoptera, Thelypteris noveboracensis, T. palustris, Dryopteris marginalis,* and *D. spinulosa.*

RESULTS

Chromatographs examined in the presence of long wave ultraviolet light in combination with ammonia vapor revealed little similarity between the Oak Ferns and the others with which they have been grouped (*Fig. 1*). The fluorescent substances were indicated by their positive reactions with alkaline silver nitrate to be phenolic in composition. One compound (labelled 5) with an rf value of 0.67 was common to all species. Several compounds appeared to be genus specific. Five compounds (labelled 4) with rf values of 0.16, 0.32, 0.40, 0.80 and 0.89 were specific to *Dryopteris marginalis* and *D. spinulosa.* Compounds (labelled 3) with rf values of 0.14, 0.38, 0.48, 0.80 and 0.89 were characteristic of *Thelypteris palustris* and *T. noveboracensis. Phegopteris* was characterized by spots (labelled 2) with rf values of 0.35, 0.49, and 0.91. Six compounds (labelled 1) with rf values of 0.15, 0.28, 0.40, 0.55, 0.78, and 0.90 were detected in *Gymnocarpium* exclusively. A few phenolics which appeared to be species specific were noted in all ferns considered.

Some chromatograms were sprayed with ninhydrin. Little could be derived concerning generic relationships, as most ninhydrin positive compounds either were shared by all ferns sampled or were species specific (*Fig. 2*).

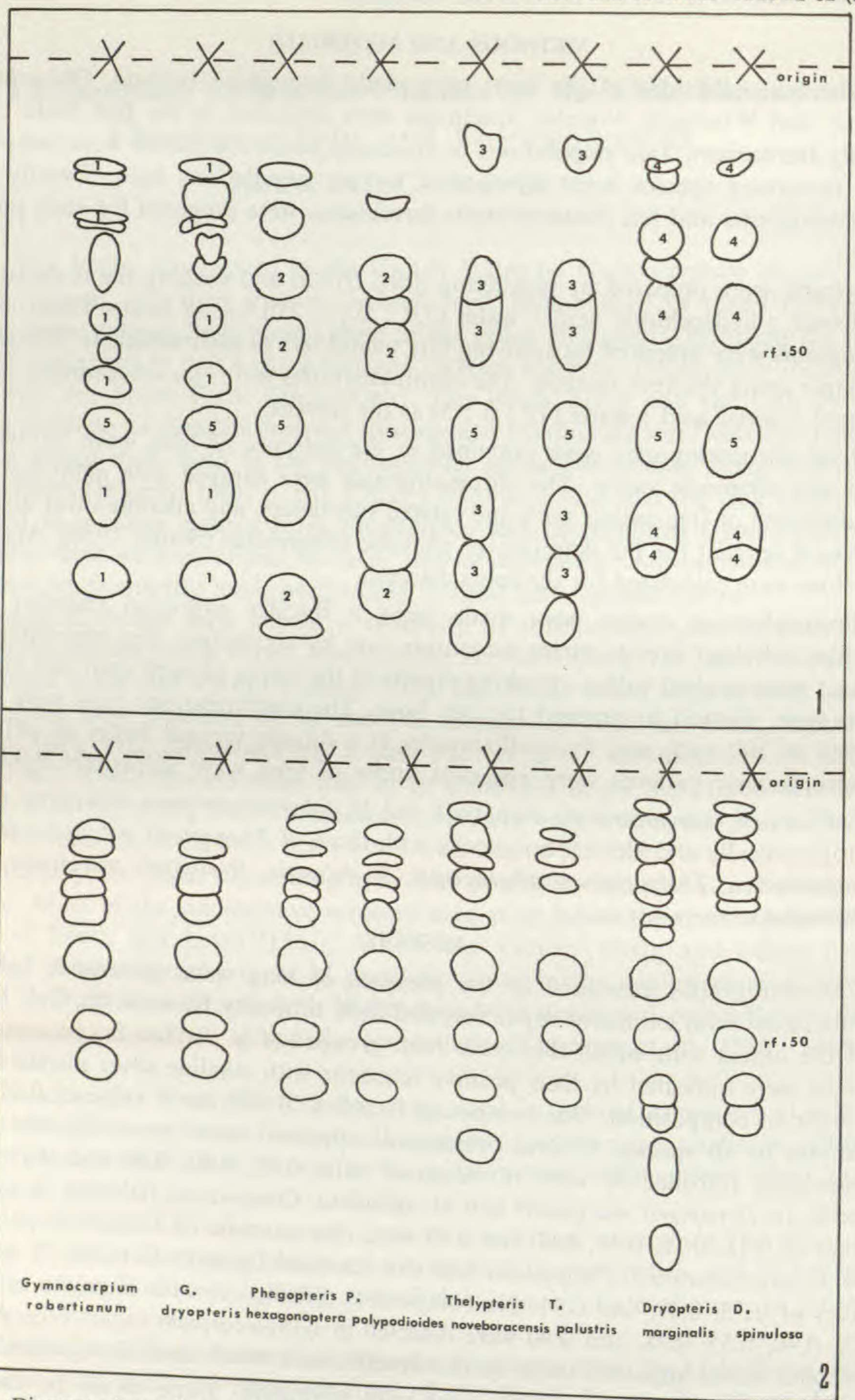

Fig. 1. Diagram of one-dimensional descending chromatograph under long wave UV and ammonia vapor, × 0.2. Fig. 2. Diagram of one-dimensional descending chromatograph under long wave UV and ninhydrin, × 0.2.

Cellulose acetate electrophoresis membranes were observed in the presence of long wave ultraviolet light (*Fig. 3*). All constituents migrated toward the positive pole. The bands were phenolic or amino acid in nature due to the method of extract preparation; however, the exact constituency of each band was not determined.

The patterns obtained were quite distinctive within each genus. *Dryopteris marginalis* and *D. spinulosa* shared green and blue fluorescent bands. Three common bands, avocado, blue-violet, and green, were noted in *Thelypteris noveboracensis* and *T. palustris*. The genus *Phegopteris* was characterized by two, specific, blue and blue-violet bands. Two additional species-specific green bands were seen in *P. polypodioides*. Distinctive blue-violet and yellow bands were seen in the Oak Ferns. These did not correspond with those of the other genera.

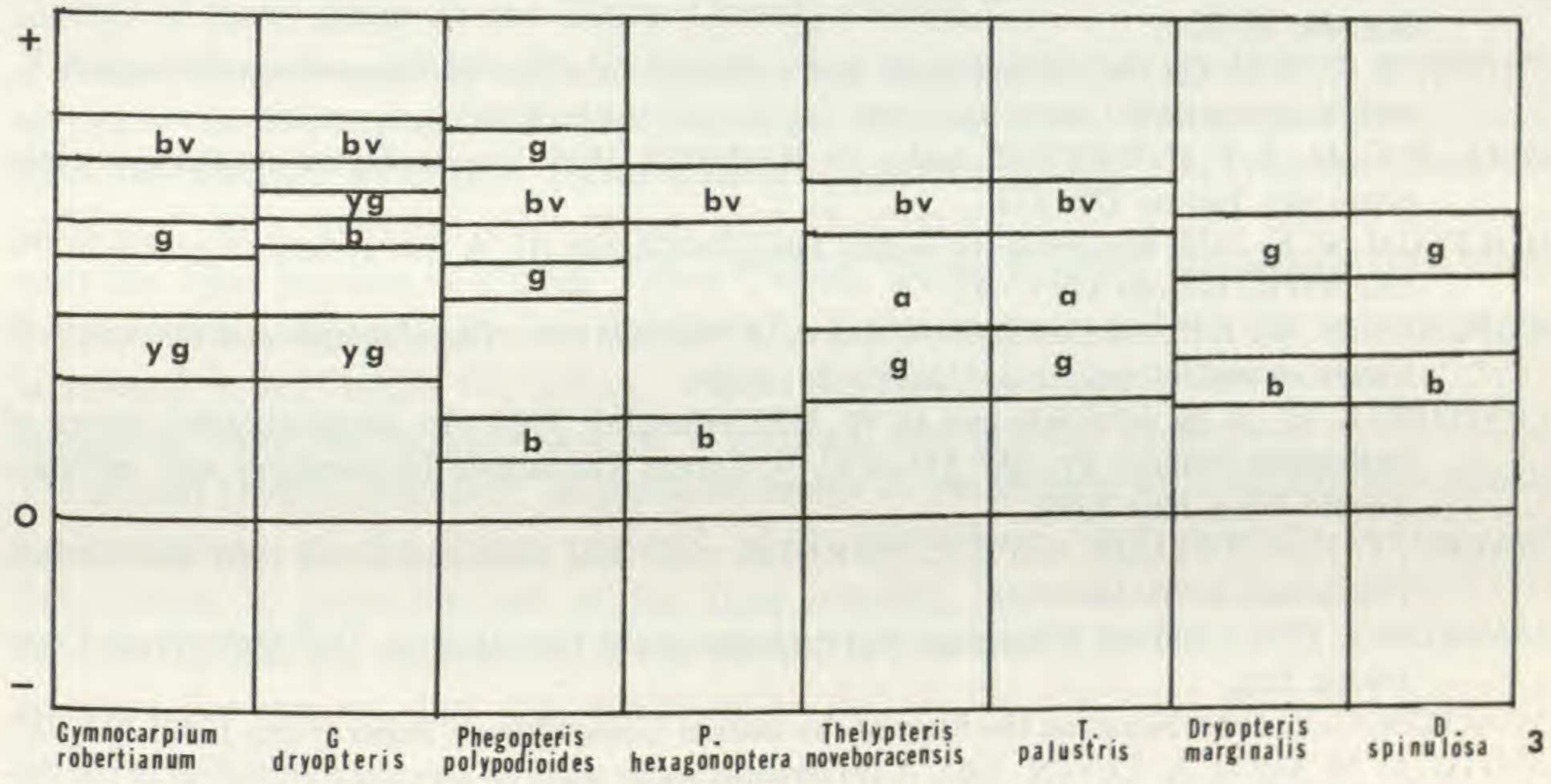

Fig. 3. Drawing of cellulose acetate electrophoresis membrane under long wave UV, × 0.35.

DISCUSSION

The application of paper chromatography in studies at the generic level appears promising. Significant affinities were not indicated in chromatographic patterns of the phenolics of the Oak Ferns with those of representatives of *Dryopteris*, *Thelypteris*, and *Phegopteris*. Free amino acid patterns were very similar, however, for all ferns sampled.

The band patterns derived by cellulose acetate electrophoresis were distinctive for each genus. Cellulose acetate electrophoresis has the advantages of ease of preparation and rapid migration. Some limitations of the method were indicated, however. Samples of the same extracts were used for both chromatography and electrophoresis. There were more compounds detected on the chromatograms than on the strips; thus, an incomplete separation and resolution by cellulose acetate electrophoresis is apparent. Further, many indicator reagents which are useful with paper cause distortion of the cellulose acetate membranes. As a means of

complete separation and identification of components the technique appears limited.

Additional studies of this nature must be done before a completely objective assessment of the validity of the methods can be attained.

Many problems of generic delimitation exist among the thelypteroid and dryopteroid ferns. Additional studies involving related species from North America and other continents (cf. Holttum, 1971) must be undertaken before a complete taxonomic understanding can be gained.

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